Iranian Journal of Veterinary Science and Technology

Received:2023- Jun-14
Accepted after revision: 2024- May-05
Published online: 2024- May-31

RESEARCH ARTICLE

DOI: 10.22067/ijvst.2024.82852.1264

Antidiabetic effects of the heat-killed *Actinomycetales* species in the liver and kidney of diabetic rats

Monireh Khordadmehr[a], Solin Ghaderi[a], Mehran Mesgari-Abbasi[b], Farinaz Jigari-Asl[a], Katayoon Nofouzi[a], Graham McIntyre[c]

[a] Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.
[b] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
[c] Center for Infectious Diseases and International Health, Windeyer Institute for Medical Sciences, University College London, UK.

ABSTRACT

Type 1 diabetes mellitus (T1DM) occurs due to the decrease in insulin secretion following the destruction of pancreatic beta cells. This disease is increasing worldwide, especially among children under the age of 5 years, which is usually associated with irreversible complications such as hepatopathy and nephropathy. The present study aimed to investigate the antidiabetic effect of the heat-killed Actinomycetales species, including Gordonia bronchialis (Gb), and Tsukamurella inchonensis (Ti) in streptozotocin-diabetic rats by oral administration. This experiment was performed in six groups, including healthy control, diabetic control, low-dose Gb (G1), high-dose Gb (G2), low-dose-Ti (T1), and high-dose Ti (T2). Subsequently; the levels of ALT, AST, total protein, albumin, BUN, creatinine, CRP, IL-1β, and IL-2 were measured in the serum samples in the 14th and 21st days. Besides, histopathological lesions were studied in the liver and kidney. Our findings showed that Gb and Ti could alter the examined serum parameters, particularly in the T2 groups. Also, histological examination revealed a remarkable attenuation in the pathological lesions such as focal necrosis, vascular congestion, and hemorrhage in the liver and kidney of the treated rats by Gb and Ti. Here, it is concluded that oral administration of the heat-killed Actinomycetales species, particularly with a high dose of Ti, could beneficially improve the progression of T1DM and its various complications, which can be used to treat T1DM in the future.

Keywords

Type 1 diabetes mellitus, Gordonia bronchialis, Tsukamurella inchonensis, hepatopathy, nephropathy

Number of Figures: 4
Number of Tables: 1
Number of References:: 26
Number of Pages: 9

Abbreviations

DM: Diabetes Mellitus
T1DM: Type 1 DM
T2DM: Type 2 DM
Gb: Gordonia bronchialis
Ti: Tsukamurella inchonensis
CRP: C- Reactive protein

Corresponding author: Monireh Khordadmehr
Email: khordadmehr@tabrizu.ac.ir
Tel: +98(41)3637-8744.

Introduction

Diabetes Mellitus (DM) is not a single disease but a general term that describes a collection of metabolic conditions, that result in high blood glucose levels due to defects in insulin function or secretion or both [1, 2]. Increasing evidence reported that it has affected approximately 285 million individuals globally, and this number is anticipated to increase to 439 million in 2030 [3], which is associated with severe and irreversible complications, such as nephropathy and hepatopathy [4]. Type 1 DM (T1DM) and Type 2 DM (T2DM) are the two primary forms of diabetes [5]. T1DM, formerly known as insulin-dependent diabetes mellitus (IDDM) [2]. The annual incidence of T1DM varies widely in different countries (from less than one person in 100,000 in Asia to more than 41 cases in 100,000 people in Europe). Children are newly diagnosed with this disease [5]. This disease is increasing worldwide, especially among children under the age of 5 years [1, 5-7]. Chemokines play a crucial role in both the immune system and inflammatory processes, which have been suggested as inducers of β-cell damage in human insulin-dependent diabetes mellitus [1].

Actinomycetales species can switch off pre-existing Th2 preponderance and stimulate Th1-mediated mechanisms. Recently, some aerobic Actinomycetales species, like Gordonia bronchialis and Tsukamurella inchonensis are capable of exerting subtly different adjuvant or immunomodulatory activities [7, 8]. In this regard, it has been revealed that subcutaneous injection of these killed bacteria improves T2DM and obesity in mice animal models [8]. Also, our previous reports presented the improvement impacts of the heat-killed Actinomycetales species in the pancreas [9], testes [10], and intestine [11] of diabetic rats. Thus, in the present study, the beneficial effects of the heat-killed Actinomycetales species, including Gordonia bronchialis (Gb) and Tsukamurella inchonensis (Ti), were investigated in streptozotocin-diabetic rats by oral administration. For this purpose; the liver and kidney biochemical indicators such as ALT, AST, total protein, albumin, blood urea, and creatinine were evaluated in the serum samples. Besides; the C-reactive protein (CRP), IL-1β, and IL-2 levels were measured and associated with histopathological evaluation of the liver and kidney.

Abbreviations Cont'd

STZ: Streptozotocin
AST: aspartate aminotransferase
ALT: alanine aminotransferase

Results

Biochemical findings

Lower levels of serum insulin along with elevated glucose values were detected in control diabetic rats compared to the other treated groups (supplementary file). Interestingly, there were no significant differences ($p > 0.05$) in glucose values between the diabetic rats and the treated groups in a dose-dependent manner. Moreover, lower insulin levels were observed in the diabetic rats, which improved significantly in the treated groups by using the bacteria, especially in Ti-recipient groups (Figure supplementary 1).

The marked decreased values of serum albumin and total protein (figure 1A, B) were assessed in the diabetic animals when compared with other groups, which improved beneficially in all diabetic-treated groups. In albumin measurement, there were notable differences in healthy rats with other experimental groups, and also a marked difference ($p < 0.05$) was noted among the low-dose and high-dose Gb recipient groups on the 14th and 21st sampling days. In total protein data, both low and high-dose Gb and Ti recipient groups showed significant differences ($p < 0.05$) with the healthy and diabetic animals. Notably, the highest levels of both albumin and total protein were observed in the T1, G2, and G2 groups, on the 7th, 14th, and 21st sampling days.

Significantly decreased levels of blood urea and creatinine (figure 1C, D) ($p < 0.05$) were observed in all diabetic-treated groups as compared with the diabetic group. The significant difference in low-dose and high-dose groups was only in urea values on the 14-sampling day.

The activities of AST and ALT diminished in the diabetic rats (figure 1E, F) when compared with the healthy and diabetic-treated rats, particularly in Gb-recipient groups in a dose-dependent manner ($p < 0.05$).

CRP, IL-1β, and IL-2 serum levels

Here, remarkably higher levels of IL-1β and CRP inflammatory cytokines were found within the diabetic rats as compared to healthy animals, which improved in a dose-dependent manner in all diabetic-treated groups (figure 1G, H). On the other hand, considerably lower levels of IL-2 were observed in the diabetic animals when compared with the healthy rats. The serum levels of IL-2 significantly increased and improved in all diabetic-treated groups without a dose-dependent manner between Gb-recipient and Ti-recipient groups.

Histopathological findings

In the liver, healthy control rats presented a nor-

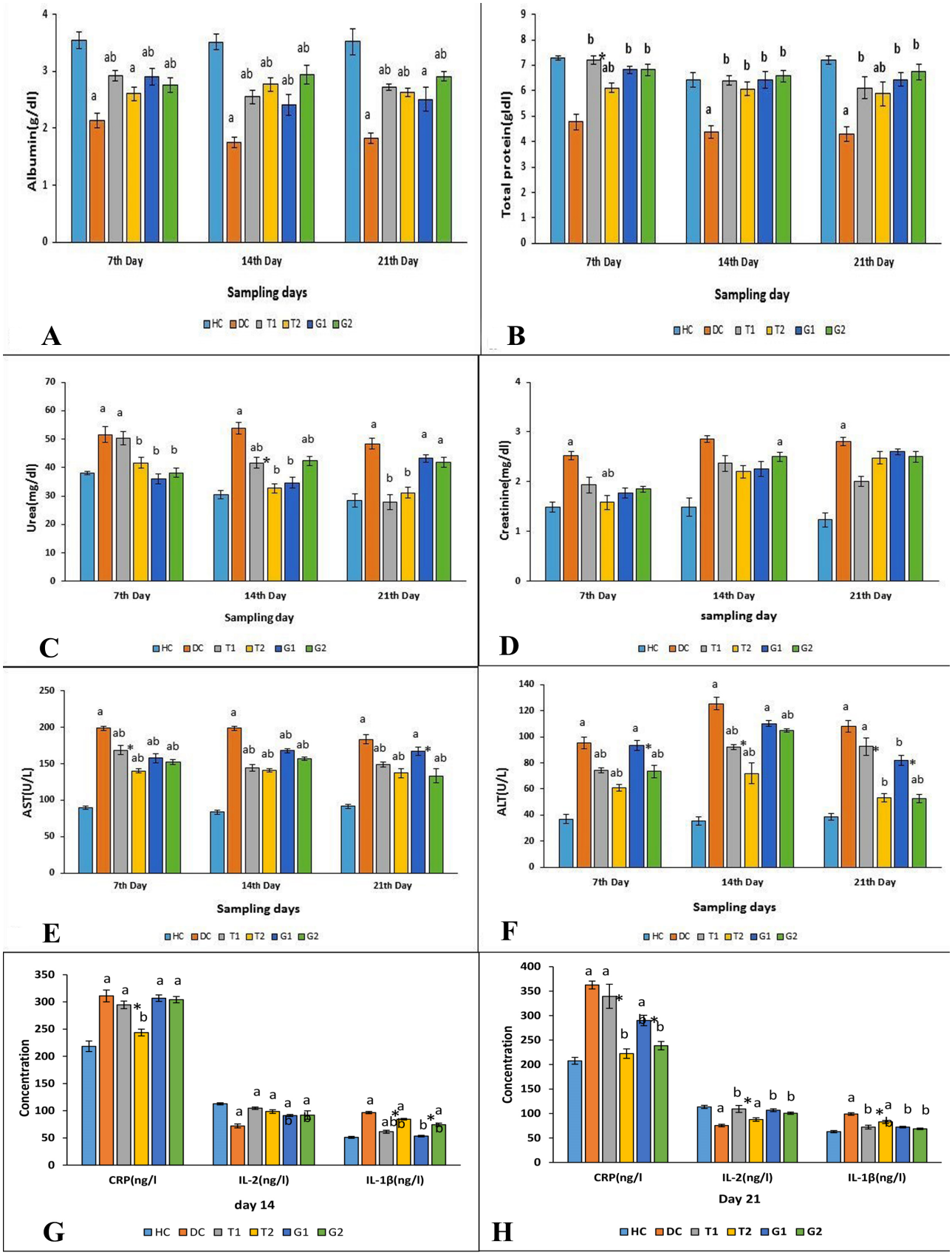

Figure 1.
The effects of oral administration of Actinomycetales species on the serum levels of albumin (A), total protein (B), urea (C), creatinine (D), AST (E), ALT (F), CRP/Il-2/IL-1β/day 14 (G), and CRP/Il-2/IL-1β/day 21 on STZ-induced diabetes. Data are presented as the mean ± SD. Differences were considered significant with $p < 0.05$. a: a significant difference with healthy control (HC); b: a significant difference with diabetic control (DC); *: a significant difference between low-dose and high-dose treated groups.

mal tissue structure consisting of evenly arranged polyhedral hepatocytes radiating outward from the central vein to the periphery. By contrast, there were severe to moderate pathological changes in the control diabetic group, including cell swelling and vacuolar degeneration of hepatocytes, particularly around the central veins, dilatation, and congestion of sinusoids, congestion in the central veins, focal single-cell necrosis, and mild hepatitis. Surprisingly, the livers of the animals in T1, T2, G1, and G2 groups exhibited marked improvements in all of the histopathological features (figure 2), particularly in the Ti high dose recipient group in the 21st after treatment with mild hepatocyte degeneration and vascular congestion.

In the kidney, a normal renal parenchymal structure (figure 3), together with well-defined glomeruli and tubules, was observed in the healthy control rats. In contrast, the diabetic animals with no treatment presented severe to moderate pathological changes comprising tubular epithelium degeneration, vacuolization and single-cell necrosis, vascular congestion, focal hemorrhage, focal interstitial nephritis, and atrophy with the congestion of glomeruli. Interestingly, all treated groups showed significant improvements in the renal lesions, mainly at each of both doses of Gb, which presented only mild vascular congestion and tubular hyaline casts.

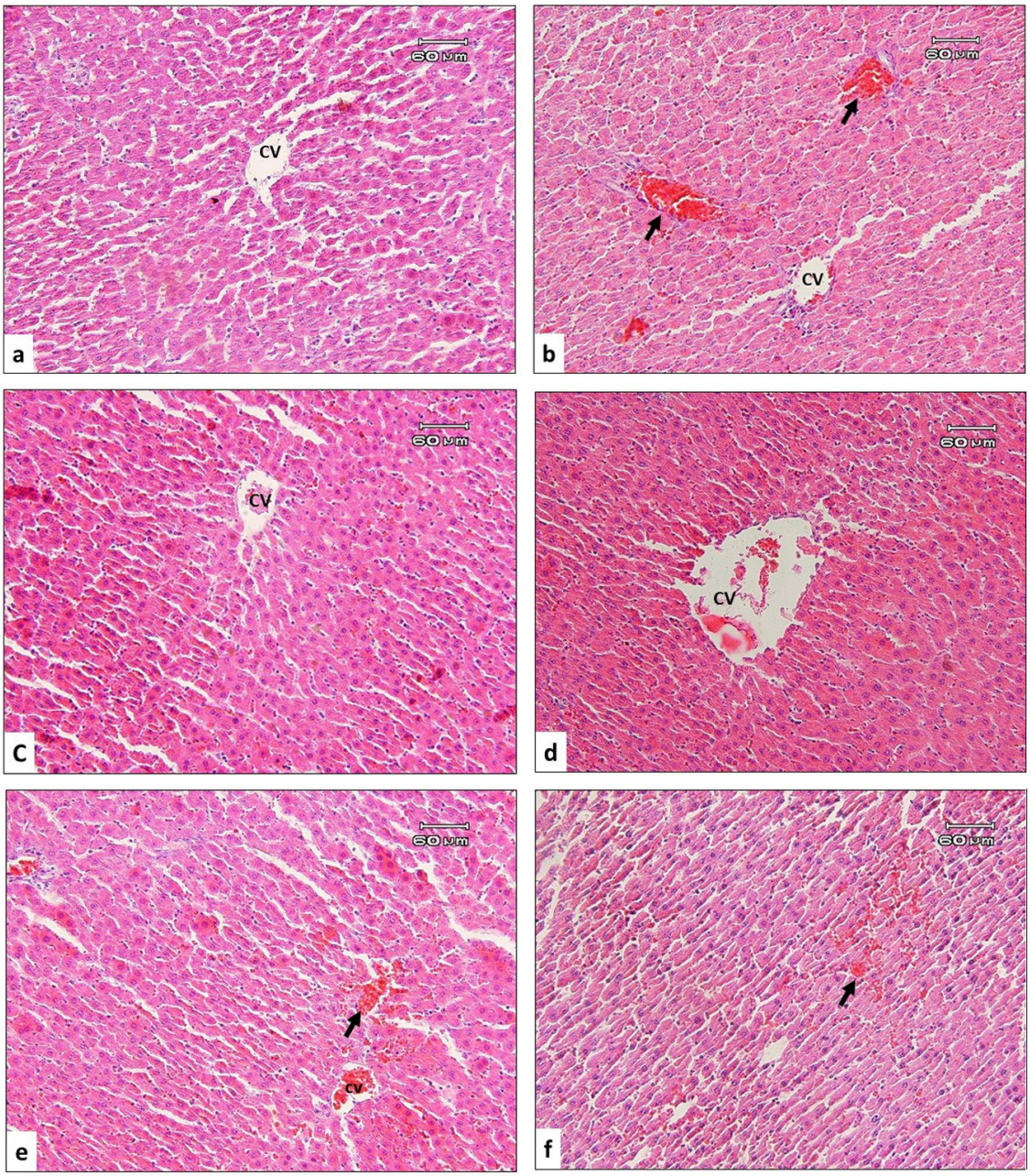

Figure 2.
Liver, rat, STZ-induced diabetes. a: healthy control with a normal liver structure; b: diabetic control with severe cell swelling and hemorrhage (arrows); c: high dose Ti-recipient group (T2) with mild cell swelling; d: low dose Ti- recipient group (T1) with mild to moderate cell swelling; e: low dose Gb- recipient group (G1) with mild to moderate cell swelling and hemorrhage (arrows); f: high dose Gb-recipient group (G2) with mild cell swelling and focal hemorrhage (arrow). H&E.

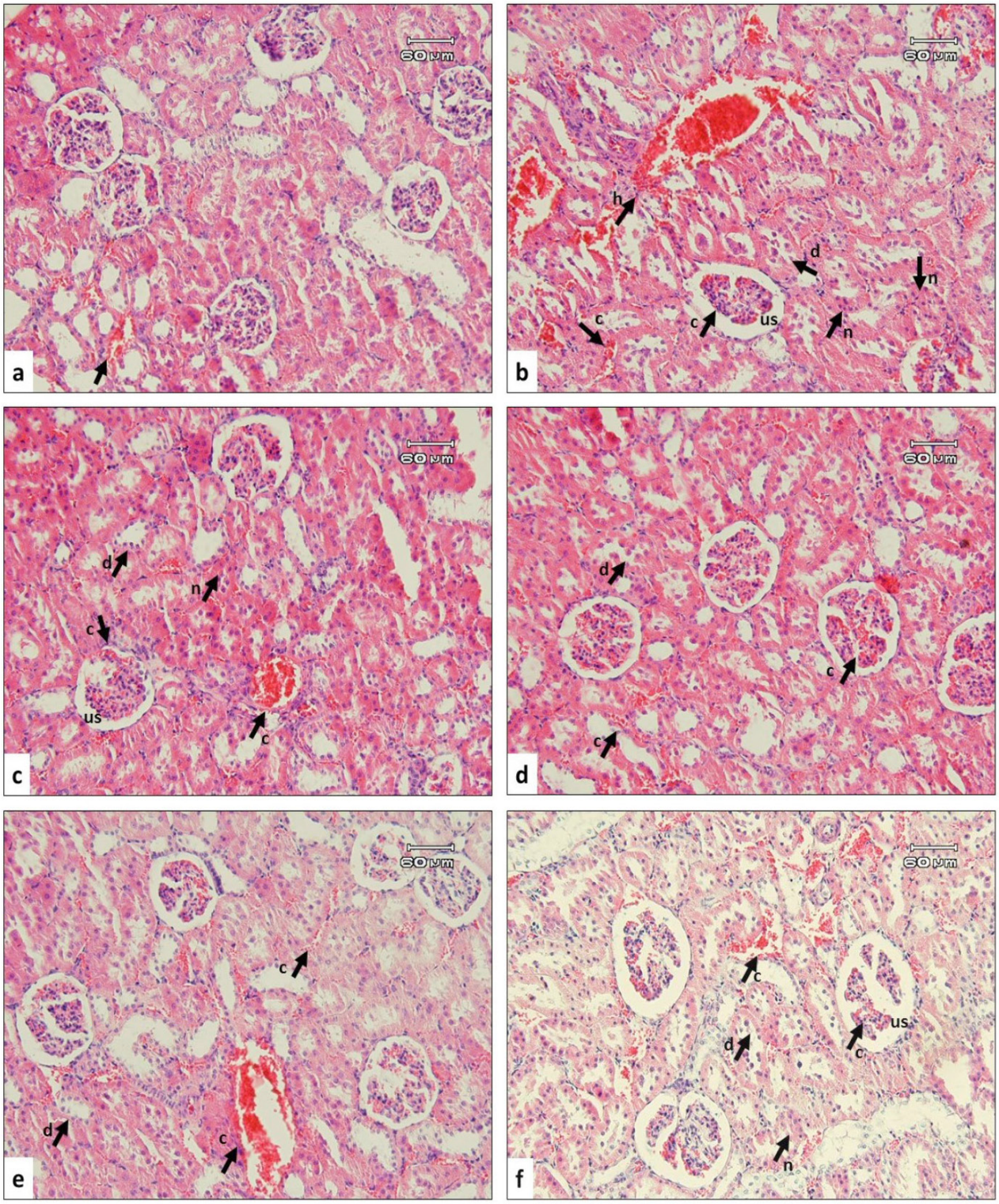

Figure 3.
Kidney, rat, STZ-induced diabetes. a: healthy control with a normal renal parenchymal structure; b: diabetic control showed severe to moderate tubular epithelium degeneration (d), vacuolization and cell necrosis (n), congestion (c) and hemorrhage (h) associated with enhancement of urinary space (us); c: low dose Gb-recipient group (G1) with mild congestion; d: high dose Gb- recipient group (G2) with mild congestion; e: low dose Ti- recipient group (T1) with mild to moderate congestion and cellular degeneration (arrows); f: high-dose Ti-recipient group (T2) with mild to moderate congestion and cellular degeneration (arrows). H&E.

Discussion

MSCs can differentiate into other cells and secrete or suppress the growth hormones or essential cytokines in the wound environment. AD-MSCs in large quantities are easily isolated and cultured and have great potential in therapeutic applications [20]. In the present study, the regeneration of the epithelium was completed on day 3 in the hydrogel+MSCs group, and in the other groups, it was completed on day 10. In different studies, the start of epithelialization was differ-

ent, and most of them had significant differences with the control group. It has been shown in a research that AD-MSCs increase blood supply and the rate of granulation tissue formation in wounds, survive in the wound for up to 14 days, and have lasting effects on the wound [21].

Contrary to the above studies, one investigation showed that between the control and treatment groups, there was no significant difference in terms of the amount of collagen, epithelialization, angiogenesis, and number of fibroblasts and macrophages. They showed that AD-MSCs had a significant effect in reducing the size of the wound, but their effect on the severity of skin lesions and pathological factors was not confirmed. Compared to BMSCs (Bone MSCs), they have a lower ability to differentiate into endothelial cells [22]. Furthermore, Karimi et al.(2014) reported that AD-MSCs had no significant improvement in acute burn wound healing [23].

The present study showed that the hydrogel+MSCs group had the highest amount of granulation tissue and angiogenesis on days 3 and 10, which decreased on day 21. Lotfi et al. (2019) stated that the granulation tissue thickness in the keratinocyte/MSCs/scaffold group rose in the first week, and declined significantly in the second week compared to other groups [24]. In the present study and the research by Lotfi et al., polymers made from natural materials such as hydrogels provided a suitable environment and direct cell contact. In the current investigation, the hydrogel group had the highest amount of granulation tissue on day 21 compared to the hydrogel+MSCs group, which could indicate the critical role of stem cells that have paracrine signaling properties, which reduce inflammation, and promote angiogenesis and cell proliferation [24].

In this study, we used allogenic AD-MSCs. According to the literature, autologous MSCs have more accelerated cicatrization than allogeneic MSCs. However, in burn injuries, allogeneic MSCs can be the only available option [25]. Research showed that the intradermal injection of allogenic AD-MSCs in burn wounds caused a significant difference on the 14th days with the control group [16].

In the detection of the SYR gene by PCR, the band of this gene was observed only on day 3 in the hydrogel+MSCs group. Hanson et al. (2016) injected allogenic male AD-MSCs intradermally in the partial-thickness of female minipigs. In female tissues, male DNA content was evaluated by the PCR amplification of a 377 bp segment from chromosome Y. They observed Y chromosome bands with a decreasing trend on days 0, 7, and 10 [26]. Based on this study, it would have been better to include the cell tracking investigations of day 7 in the present study to better understand this decreasing process . The difference between the present study and the above study may be attributed to the type of animal modeling, method, and amount of stem cell injection. The reason for the decrease in the presence of MSCs in the wound site is unknown. However, it can be because of MSCs migration from the wound site, MSCs phagocytosis by macrophages, or mechanisms involved in cell processing and tissue regeneration [26].

Tragacanth gum hydrogel can be a suitable scaffold for AD-MSCs. It accelerates the proliferation and differentiation of cells and provides a suitable space for the support and adhesion of cells. It is also capable of expressing genes for up to 21 days and maintains the original morphology of cells. In the present study, the hydrogel and control groups had significantly different epithelialization on day 10. Although no significant difference was observed in inflammation and granulation tissue formation between the hydrogel and other groups, on the 10th and 21st days, the rate of granulation tissue formation in the hydrogel group was higher than in other groups. One of the reasons is the high concentration of hydrogel, and the reduction of inflammation in the stem cell group is may result from the presence of MSCs. A study similar to the current research showed that wound closure occurs faster in the PCL-GT-stem cells group than in the PCL-GT scaffolds group. Granulation tissue, collagen synthesis, and angiogenesis were improved in the PCL-GT-stem cells group. They stated that GT accelerates the transition from the inflammatory and germinal phases as well as the maturation of scar tissue [27–29]. Researchers demonstrated that creams made from Tragacanth gum at a concentration of 6% had the highest effect on rabbit wound healing compared to the control group [27]. In another study, with the daily application of Tragacanth gel, a significant difference was observed in terms of epithelialization, inflammation, and granulation tissue on the 10th day compared to the control group. Similar to the present study, they showed that Tragacanth gum caused most of the wounds to close on the 10th day by accelerating wound contraction [28].

A proper dressing should enhance epithelial regeneration, control the amount of exudate, prevent material leakage, reduce inflammation and infection, and be comfortable for the patient. In this study, amniotic membranes, as an economically reasonable alternative biomaterial were used to benefit from the above characteristics and also prevent hydrogel leakage. In some studies, the use of amniotic membranes alone in wound healing was ineffective [30], but in others, it had no significant difference with the control group or other treatment groups [31]. Studies

have shown that using the amniotic membrane alone is effective for shallow wounds while a more effective solution is required in wide and deep wounds, such as full-thickness wounds and third-degree burns [32].

Studies showed that the application of MSCs with cellular/acellular amniotic membrane multiplies the rate of wound healing compared to utilizing amniotic membrane alone [33]. In this study, on the 3rd days in the stem cell group, acellular human amniotic membrane was observed as a serous layer covered with squamous cells on the wound scab, which is a sign of the effective role of MSCs.

In summary, in this study, the synergism effect of MSCs, Tragacanth gum hydrogel, and human amniotic membrane as a dressing was investigated. Histopathology results showed that the combination of SCs and Tragacanth gum hydrogel was influential in the immediate wound closure, and the human amniotic membrane played a supporting role.

Materials and Methods

Ethical approval

The experiment was authorized by the Research Ethics Committee, Tabriz University of Medical Sciences, Iran (ethical approval code: 5-4-1171).

Experimental design

Sixty healthy adult male Wistar rats weighing approximately 245–365g, were obtained and divided equally into six groups (Table 1). In five groups, T1DM was induced by an intraperitoneal (i.p) injection of Streptozotocin (STZ) (Sigma Aldrich Co.-USA) with a dosage of 55 mg/kg. Blood glucose levels were assessed three days later, the time point when treatments were initiated [9-11]. The treatments were managed according to Table 1 by two different doses (low dose and high dose) of two of the heat-killed Actinomycetales species, including G. bronchialis (Gb) and T. inchonensis (Ti), and also normal saline (for the diabetic and healthy control groups) [8-11], which was administered orally applying intragastric gavage technique for 14 consecutive days. The animals were monitored daily for 21 days. Blood specimens were collected after anesthesia (by i.p administration of 50 and 8 mg/kg BW of ketamine and xylazine, respectively) on the 7th, 14th, and 21st days. Sera were discreet at 750 × g for 15 min for upcoming biochemical and immunological assessments. Besides, five rats in each group were euthanized, and tissue specimens from the liver and kidney were collected for histopathological examination, which was fixed in 10% buffered formalin.

Biochemical assays

Serum biochemical indicators assessment

All of the examined biochemical indicators, such as blood glucose levels and serum insulin values, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), blood urea, and creatinine, albumin, and total protein were evaluated on the 7th, 14th and 21st sampling days of sampling by commercially available kits following the manufacture's instructions (Pars Azmoon, Tehran, Iran) and using a spectrophotometer (Photometer 5010, Berlin, Germany). The activities of AST and ALT were evaluated by a modified method of Reitman-Frankel at 340 nm [24]. The measurement of blood urea and creatinine was performed based on the methods of diacetyl monoxime (546 nm) and Jaffe (500 nm), respectively [24]. Besides, the evaluation of serum albumin and total protein was performed according to the methods of bromocresyl green (546 nm) and biuret (580 nm), respectively [24].

IL-1β, IL-2 and CRP evaluation

The levels of IL-1β, IL-2, and CRP were assessed in the preserved serum samples on the 14th and 21st sampling days using Rat ELISA commercial kits (Koma Biotech, Korea) following the manufacturer's instructions [25].

Histopathological examination

The formalin-fixed tissue samples underwent standard processing, sectioned, and stained with common hematoxylin and eosin (H&E), and then studied microscopically under a light microscope (CH-3, Olympus, Japan). The tissue sections were examined for pathological changes such as atrophy, necrosis, vascular congestion, and hemorrhage [26].

Statistical analysis

The provided data were analyzed using SPSS software (SPSS, version 16 for Windows, USA). More specifically, the ANOVA and non-parametric tests were employed to statistically analyze the serum parameters and pathological lesions across the different groups, respectively, and a $p < 0.05$ was deemed significant.

Table 1.
Different treatments were conducted in six groups of 10 rats each in the present study.

groups	Treatment for 14-continuous days
Low dose Gb	Diabetes treated with 105 CFU/rat* *G. bronchialis*
High dose Gb	Diabetes treated with 107 CFU/rat *G. bronchialis*
Low dose Ti	Diabetes treated with 105 CFU/rat *T. inchonensis*
High dose Ti	Diabetes treated with 107 CFU/rat *T. inchonensis*
Diabetic control	Diabetes treated with normal saline
Healthy control	No diabetes treated with normal saline

*CFU/rat: Colony Forming Unit).

Authors' Contributions

M. Kh. and M.M.A.: Conceptualization, Methodology, Writing - Review & Editing. K.N., and G.M.I.: Conceptualization and Methodology. S. Gh. And F.J.A.: Investigation, Writing - Original Draft. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Acknowledgements

The authors are thankful for the financial support of the University of Tabriz, Iran and the Drug Applied Research Center, Tabriz University of Medical Sciences, Iran.

Competing Interests

The authors have no financial conflicts of interest.

References

1. Dogan Y, Akarsu S, Ustundag B, Yilmaz E, Gurgoze MK. Serum IL-1β, IL-2, and IL-6 in insulin-dependent diabetic children. Mediators of inflammation. 2006;2006. Doi:10.1155/MI/2006/59206

2. Russell MA, Morgan N. The impact of anti-inflammatory cytokines on the pancreatic β-cell. Islets. 2014;6(3):e950547. Doi:10.4161/19382014.2014.950547

3. Blake R, Trounce IA. Mitochondrial dysfunction and complications associated with diabetes. Biochimica et Biophysica Acta (BBA)-General Subjects. 2014;1840(4):1404-12. Doi:10.1016/j.bbagen.2013.11.007

4. Sayin N, Kara N, Pekel G. Ocular complications of diabetes mellitus. World journal of diabetes. 2015;6(1):92. Doi:10.4239/wjd.v6.i1.92

5. Samadi N, Allahyari I, Zamanzadeh V, Dadkhah B, Mohammadi M. Educational Points for Prevention of Type 1 Diabetes and its Complications: A Systematic Review. J Clin Cell Immunol S. 2012;2:2.

6. Derosa G, Cicero AE, Bertone G, Piccinni MN, Ciccarelli L, Roggeri DE. Comparison of fluvastatin+ fenofibrate combination therapyand fluvastatin monotherapy in the treatment of combined hyperlipidemia, type 2 diabetes mellitus, and coronary heart disease: a 12-month, randomized, double-blind, controlled trial. Clinical therapeutics. 2004;26(10):1599-607.

7. Hansrani M, Stanford J, McIntyre G, Bottasso O, Stansby G. Immunotherapy for the prevention of myointimal hyperplasia after experimental balloon injury of the rat carotid artery. Angiology. 2010;61(5):437-42. Doi:10.1177/0003319710366128

8. Tarrés MC, Gayol MdC, Picena JC, Alet N, Bottasso O, McIntyre G, et al. Beneficial effects of immunotherapy with extracts derived from Actinomycetales on rats with spontaneous obesity and diabetes. Immunotherapy. 2012;4(5):487-97. Doi:10.2217/imt.12.37

9. Khordadmehr M, Ghaderi S, Mesgari-Abbasi M, Jigari-Asl F, Nofouzi K, Tayefi-Nasrabadi H, et al. The Beneficial Effects of Actinomycetales Immune Modulators in the Pancreas of Diabetic Rats. Advanced Pharmaceutical Bulletin. 2021;11(2):371. Doi:10.34172/apb.2021.035

10. Khordadmehr M, Ghaderi S, Abbasi MM, Nofouzi K, McIntyre G. The improvement effects of Gordonia bronchialis on male fertility of rats with diabetes mellitus induced by streptozotocin. Pharmaceutical Sciences. 2019;25(3):227-34.

11. Mesgari-Abbasi M, Ghaderi S, Khordadmehr M, Nofouzi K, Tayefi-Nasrabadi H, McIntyre G. Enteroprotective effect of Tsukamurella inchonensis on streptozotocin induced type 1 diabetic rats. Turkish Journal of Biochemistry. 2019;44(5):683-91.

12. Hassanalilou T, Payahoo L, Shahabi P, Abbasi MM, Jafar-abadi MA, Bishak YK, et al. The protective effects of Morus nigra L. leaves on the kidney function tests and histological structures in streptozotocin-induced diabetic rats. Biomed Res. 2017;28(14):6113-8.

13. Zafar M, Naqvi SN-u-H, Ahmed M, Kaimkhani ZA. Altered Liver Morphology and Enzymes in Streptozotocin Induced Diabetic Rats. International journal of morphology. 2009;27(3).

14. Arkkila PE, Koskinen PJ, Kantola IM, Rönnemaa T, Seppänen E, Viikari JS. Diabetic complications are associated with liver enzyme activities in people with type 1 diabetes. Diabetes Research and Clinical Practice. 2001;52(2):113-8. Doi:10.1016/s0168-8227(00)00241-2

15. Edet E, Atangwho I, Akpanabiatu M, Edet T, Uboh F, David-Oku E. Effect of Gongronema latifolium leaf extract on some liver enzymes and protein levels in diabetic and non diabetic rats. J Pharm Biomed Sci. 2011;1(5):104-7.

16. Özer G, Teker Z, Cetiner S, Yılmaz M, Topaloglu AK, Önenli-Mungan N, et al. Serum IL-1, IL-2, TNFα and INFγ levels of patients with type 1 diabetes mellitus and their siblings. Journal of Pediatric Endocrinology and Metabolism. 2003;16(2):203-10.

17. Tomoda T, Kurashige T, Taniguchi T. Imbalance of the interleukin 2 system in children with IDDM. Diabetologia. 1994;37:476-82. Doi:10.1007/s001250050135

18. Wagner R, Bonifacio E, Bingley P, Genovese S, Reinwein D, Bottazzo G. Low interleukin-2 receptor levels in serum of patients with insulin-dependent diabetes. The clinical investigator. 1994;72:494-8. Doi:10.1007/BF00207476

19. Karlsson Faresjö M, Ernerudh J, Ludvigsson J. Cytokine profile in children during the first 3 months after the diagnosis of type 1 diabetes. Scandinavian journal of immunology. 2004;59(5):517-26. Doi:10.1111/j.0300-9475.2004.01420.x

20. Abbas MA, Abraham D, Kushner JP, McClain DA. Anti-obesity and pro-diabetic effects of hemochromatosis. Obesity. 2014;22(10):2120-2. Doi:10.1002/oby.20839

21. Davì G, Chiarelli F, Santilli F, Pomilio M, Vigneri S, Falco A, et al. Enhanced lipid peroxidation and platelet activation in the early phase of type 1 diabetes mellitus: role of interleukin-6 and disease duration. Circulation. 2003;107(25):3199-203. Doi:10.1161/01.CIR.0000074205.17807.D0

22. Erbağci AB, Tarakçioğlu M, Coşkun Y, Sivasli E, Namiduru ES. Mediators of inflammation in children with type I diabetes mellitus: cytokines in type I diabetic children. Clinical biochemistry. 2001;34(8):645-50. Doi:10.1016/s0009-9120(01)00275-2

23. Khattab MH, Shahwan MJ, Hassan NAGM, Jairoun AA. Ab-

normal High-sensitivity C-reactive Protein is Associated with an Increased Risk of Cardiovascular Disease and Renal Dysfunction among Patients Diagnosed with Type 2 Diabetes Mellitus in Palestine. Review of Diabetic Studies. 2022;18(1):27-33.

24. Tietz NW. Clinical guide to laboratory tests. Clinical guide to laboratory tests1995. p. 1096-.

25. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman J, Smith JA, et al. Short protocols in molecular biology. New York. 1992;275:28764-73.

26. Klopfleisch R. Multiparametric and semiquantitative scoring systems for the evaluation of mouse model histopathology-a systematic review. BMC veterinary research. 2013;9:1-15. Doi:10.1186/1746-6148-9-123

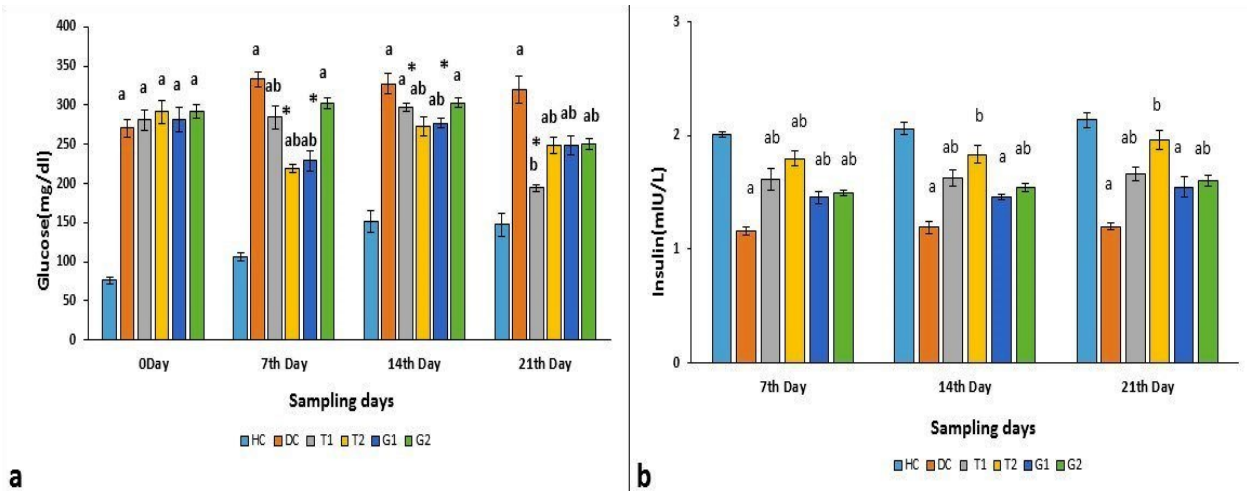

Figure S1.
The effects of oral administration of Actinomycetales species on the serum levels of glucose (A) and insulin (B) on STZ-induced diabetes. Data are presented as the mean ± SD. Differences were considered significant with $p < 0.05$. a: a significant difference with healthy control (HC); b: a significant difference with diabetic control (DC); *: a significant difference between low-dose and high-dose treated groups.

How to cite this article
Khordadmehr M, Ghaderi S, Mesgari-Abbasi M, Jigari-Asl, F, Nofouzi K, McIntyre G. Antidiabetic effects of the heat-killed *Actinomycetales* species in the liver and kidney of diabetic rats. Iran J Vet Sci Technol. 2024; 16(2): 26- 34.
DOI: https://doi.org/10.22067/ijvst.2024.82852.1264
URL:https://ijvst.um.ac.ir/article_45170.html